CARDIOVASCULAR DISEASE RESEARCH

Dr. David Robinson

DR. ROBINSON: I am going to talk about cardiovascular disease research. And by cardiovascular disease, I refer to the blood-carrying vessels of the circulation and the heart and to all of the diseases that are found in that particular system. The arteries that carry oxygen-rich blood from the lungs to the tissues and the veins that carry back blood that has given up its oxygen and taken on carbon dioxide to be returned to the lungs for expiration in breath. And all of that driven by this lump of muscle in the middle of the chest called the heart. A very strong pump which often these days, unfortunately, goes wrong.

Now there are very many conditions associated with this system, and obviously, I have to focus on some of them rather than deal with the whole panolply of cardiovascular disease. I will not be dealing, for example, with defective rhythms in heart except only very peripherally. I am really going to concern myself with the two largely killing diseases: atherosclerosis, sometimes called hardening of the arteries, and hypertension, or high blood pressure.

What I will do is briefly deal with the major cardiovascular afflictions that we suffer from before going on to describe some of the ways in which modern research is trying to deal with this situation which is, in fact, the leading cause of death in America beyond any cancer, beyond any accidents, beyond anything else. Cardiovascular disease is still the leading cause of death.

We will deal with the blood vessels, as I say, which convey blood driven by this pump in the middle of the chest. Here is a cartoon of the heart. Just let me remind you that the right side of the heart deals with sending blood to the lungs, and the blood is then returned to the left side, and driven by this large muscle—the left ventricle—all around the body through the major blood vessels.

In dealing with the cardiovascular system, we are particularly interested, I think, in a special set of blood vessels that provide blood to the heart itself. This is an x-ray photograph of a living heart, and it is from a patient who has received a contrast medium, which has enabled the physician to outline very clearly this web of vessels that extend all over the surface of the heart.

These are the coronary arteries and with coronary artery disease, which you often hear about, these are the vessels that are culprits, and we will go into what kinds of things happen there. Bear in mind these vessels that supply blood, and therefore oxygen, to the

heart are the crucial parts of the circulation that give us the most trouble.

There has been a recent change in our view of blood vessels. As recently as just a few decades ago, we used to think of them merely as pipes. As Shakespeare put it, "These pipes and these conveyancies of our blood." And a very simple and not very effective view of blood vessels it was, as shown here.

Nowadays we know a lot more about blood vessels and about the inordinate complexity that they represent. They have their own muscles. They have their own nerves. They have their own blood vessels. They are very active. They are very dynamic parts of the system, and they are lined with a particular group of cells, the vascular endothelium, which has all kinds of special properties, not least of which is enabling blood to flow past at times quickly, at times slowly, and to sustain patency of the whole vessel.

We do know, too, that a principal problem that arises, in modern Western society in particular, is that of atherosclerosis, where over the years there is an accumulation of material in this area of the blood vessel, around and below the endothelium. Red here is representing flowing blood in this particular cartoon. A gradual accumulation of fatty deposits, including cholesterol and other types of material, gradually comes to narrow down the opening so that in some instances it is so narrow that hardly any blood can flow. If this cartoon was of a coronary artery supplying blood to the heart, then downstream of this particular segment, there would be very little blood flowing; the heart muscle would be starved of oxygen; and the person with such a poor blood supply would probably experience chest pain. If these vessels become totally blocked then we move to another situation that we call a heart attack.

We hear about cholesterol, particularly “bad” cholesterol and fats, which are deposited in the walls of the blood vessels, but the situation is not quite as simple as that. It is a very complex process involving all kinds of different cells and different kinds of chemicals, tissues, and all sorts of biochemical processes that are working away in the walls of the blood vessels, probably starting, yes, even in the fetus.

From very early age onwards, the development of atherosclerosis, which especially blocks the arteries supplying the heart, seems to follow an inexorable course in those predisposed to the disease. It is in these individuals that fatty deposits appear to be most damaging. But I want to emphasize that it is not just the deposition of fat. It is a very complex process involving the immune system and other kinds of mechanisms.

Eventually, as I pointed out, it may come to the crucial situation of a heart attack. This is a cross-section through a coronary artery—greatly magnified—of a patient who died from a heart attack as a result of a clot forming in the narrow area that has been left for blood flow. The atherosclerotic plaque, as it is called, has occluded most of the vessel following which, in a complex sequence of events, clots form and completely block the vessel altogether.

Downstream of this particular clot in the heart, a "coronary thrombus," there will be no blood supply to the heart, and the heart muscle will suffer accordingly. It may die. It may be sufficiently extensive, if this was a major vessel, that the patient dies with it.

Here is another cartoon to illustrate what happens. Here is a coronary artery that becomes totally occluded, and downstream there is a large area of the muscle of the heart which is now no longer provided with oxygen and will die. You can imagine if this is very, very extensive then the whole action of the heart is compromised, and this is when a heart attack becomes lethal. But many people survive their heart attacks, as we know, and that is for a variety of reasons which we will get into in a moment.

There is another phenomenon we are concerned with in the blood vessels, and that is hypertension or high blood pressure. First measured in the artery of the neck of a horse back in the 1700s in England, where the pressure of the blood forced into the vessels by the heart raises the liquid blood up to a particular level that can be measured. Nowadays, we measure blood pressure in millimeters of mercury. That is, the force exerted by the blood to produce a certain height in a mercury column.

We tend to think of blood pressure as having a critical value, below which it is safe and above which is dangerous. We often hear terms like 120/80 or 140/90. The first number refers to the pressure in the blood when the heart is beating and the second number to when the heart is relaxed. Well, it turns out that if you examine a large number of people—a huge population of people—you find blood pressures in a so-called normal distribution. We are looking here at the first number only, the systolic blood pressure. And you can see that in this particular population some have fairly low systolic pressures. The majority have them in the range of 120 to 140 and then fewer and fewer have very high numbers.

There is a continuous increase in problems as that pressure increases. It is not as if there is no mortality or morbidity up to a certain point and then all of a sudden it shoots up as the blood pressure reaches a certain level. There is just a continuum. And so on the

basis of that you might suggest that the lower the blood pressure the better. By and large, that is correct.

We have to function, however, and we have to have tests that tell us something, and by and large we determine by public health observations that if patients keep their blood pressures below or in the vicinity of 140/90 then they usually do quite well.

However, one of the principal outcomes of uncontrolled high blood pressure is brain attack or stroke. In this case, the damage is done to the brain rather than the heart and in this particular animal model the result of one side of the brain having been damaged by excessive bleeding is that the animal becomes paralyzed. That again, of course, can be a killer depending on the extent of the stroke and the damaged brain tissue. There is in many cases opportunity for recovery, but it is still a very serious problem.

And so we are dealing, by and large, with atherosclerosis, which has as its target organ the heart, where after a small heart attack in this case a certain amount of scar tissue is left, which is not functional anymore. It does not beat like the rest of the heart muscle does, and there are consequences to that in the long term. And we are also dealing with hypertension, where the target organ is the brain, and research in these areas has been going on for quite a long time.

But those interested in atherosclerosis and those interested in hypertension have generally been aligned in this sort of fashion for quite a time now. We are realizing that that is not the way to go. We should not be considering these as entirely separate diseases, but there is some reason to suggest that they should both be considered rather as part of a whole metabolic disease state, where one manifestation is blockage of the arteries and another is high blood pressure. They are inextricably intertwined. Diabetes plays a role in this as well.

And so what we now are trying to deal with is a very complex situation indeed, where genetic factors, environmental factors, anatomical factors, and so on and so forth, all have a huge interplay in an inordinately complicated situation, which results in our heart attacks and strokes.

How have we been doing? Quite well. This is a rather old slide I am afraid, but nevertheless the trends are demonstrative of what happened back in the 1960s where the death rate from coronary heart disease and stroke, in particular, began to fall rather precipitously. Now there has been a 50 percent saving of lives from stroke by comparison

to what was happening in the '50s out here in the '80s and '90s, and coronary heart disease, has also fallen quite precipitously. Very good news indeed.

Not always the case in other countries around the world, however. The diminution in death here in the United States is quite dramatic, both in men and in women, but look at Poland, where the trends have been in the opposite direction altogether. It is difficult to say why this is happening but we do know that Polish people smoke like chimneys most of the time, and cigarette smoking is a very serious risk factor that may be involved here, although that is obviously not the only reason.

But I want to emphasize that this morning I am talking about what is happening largely in the United States. If we were to plot that observed fall away in the death rate over the years and to put in a hypothetical line of what would have been the death rate had the situation remained unchanged since 1950, this is where we would have been in the '80s and '90s. This is where we actually are. And this yellow area between the two curves, one actual and one hypothetical, means there have been seven-and-a-half million lives saved up to about 1985, which is very significant indeed. So we have some reason, I suppose, to congratulate ourselves as a society on the fact that we are dealing with this somewhat successfully.

One of the ways that this has been brought about is by improved treatment. If you can get a heart attack patient into the hospital quickly enough to receive clot-busting drugs, the clot that forms and prevents blood flow down into the heart muscle can be broken up, and blood flow can resume. Here is a picture taken by x-rays of a patient who has a very severe blockage of this coronary artery. You see there is no flow downstream at all, but when given a clot busting drug which opens up the blood vessel, lo and behold the blood begins to flow again. If you can do this quickly enough, you can save the patient. Here is another one on the other side of the picture. Blockage in this vessel, opened up immediately by use of a clot busting drug.

If you can get people into emergency care where they can receive this kind of treatment quickly enough, you can save their lives these days.

Other ways of dealing with patients in this condition are to use various forms of surgery. One is percutaneous transluminal coronary angioplasty, or "balloon" angioplasty. Another is CABG, coronary artery bypass grafting. This is a more severe intervention than angioplasty, but they have both increased in large numbers—and continue to do so as we go forward into the '90s—from back in the '80s when these were

really only a pipe dream.

PTCA or balloon angioplasty is a technique in which a catheter or a tube is threaded up through the leg into the heart arteries themselves to the point where the blockage is. A balloon, which is at the end of this wire, is inflated with inert gas, and what you can see is that this blockage, fatty deposits and so on of atherosclerosis, is forced out into the wall and an opening is made in the vessel. Obviously this can be done before a heart attack occurs and people who have symptoms of chest pain and who are diagnosed quickly can have angioplasty to prevent them from having a heart attack, but it can also be used in the course of a heart attack in certain patients. That is balloon angioplasty and that is being used rather extensively.

The trouble is that after opening up the vessel in this fashion, there is a new process of blockage, called restenosis, that often starts, and this happens rather soon. It does not involve deposits of fats so much as unusual activity in cells of the vessel wall, which start to divide and produce the same effect of narrowing down the blood vessel. So another problem arises there that has to be dealt with.

There are ways in which these are dealt with rather successfully these days by using what are called stents. These are little metal lattices that are held around the balloon, which is expanded to compress the lesions out into the vessel wall and when expanded in this way, click into position and remain as a cage which keeps the vessel open. So stents provide an added advantage to the use of balloon angioplasty.

And here are a couple of different kinds of stents.

As we look now into the late '90s and early 2000s, genetic engineering being what it is, we will be able to do all kinds of things with these stents, and attempts are now being made to grow cells on the stents that have been taken from the patient and have been genetically engineered so that they produce anticlotting substances, substances which may inhibit restenosis. Stents become more and more sophisticated and more and more effective as time goes by. That is the state of the art in balloon angioplasty. We are not quite there with seeding stents with cells yet, but we are getting very close.

And then of course, there is bypass surgery. Probably most people are familiar with this. This requires, or did so until recently, opening up the chest, stopping the heart, and either taking a piece of vein from the leg or using a mammary artery (which normally supplies blood to the breast tissue) then stitching it from this large open vessel, bypassing

the lesion which is blocking off this coronary, then stitching it in below. So now instead of the blood flowing through the coronary artery and stopping there, it now flows down the bypass graft and provides life-giving oxygen to the heart muscle at the apex.

Nowadays this is being done by miniaturizing the whole process, not opening the whole chest, not stopping the heart, but going in through a little hole in the side of the chest, using a clamp to hold the beating heart, and stitching a new bypass graft across a blockage. This approach may become an outpatient procedure before too very long. Of course, the recovery time from such minor intervention is much quicker, with much less pain. Altogether much to be desired, although at the moment only available for selected patients who have a suitable sort of anatomy, but it is coming.

So much for treatment, which to a greater or lesser extent has been responsible for the drop in the death rate from cardiovascular diseases, but there is more to it than just treatment. As Pasteur himself pointed out, prevention of the disease in the first place is probably the most important feature. When I first came to America in 1969, I lived in a house in Chevy Chase, and if anyone had run past the front of my house wearing running shorts, I would have had an apoplectic fit. Nowadays everybody is in shorts on the streets. You see bicycles and tennis rackets you hardly ever saw before. The society that we live in has changed its behavior enormously to a more healthy lifestyle. The same is going on with diet. We are more conscious of what we should be eatubg and that also, I think, has had a very profound effect on this fall in death rate from these terrible diseases. In the Institute I serve, the National Heart, Lung, and Blood Institute, we have many major national programs that carry the message out to the society we live in with regard to risk factors that can be controlled. Smoking, in particular. The rest can also be controlled with the beneficial outcome that we all seek.

Yet nevertheless, despite successes, we still find there is a peak of a half million people who die from coronary heart disease every year. It is a very, very serious and killing disease. So we have to ask ourselves, although we have done these things very well and the death rate has fallen away, we are obviously not quite doing what we should be. What is the problem?

Well, the problem is not that we have failed. We have instead become victims of our own success. With the surgery and the clot-busting drugs and the like that I have discussed, we have been able to salvage—perhaps that is not the right word for human beings—we have been able to save people from their heart attacks. They do not die from them anymore. They go on and live for much longer than they would otherwise but with

damaged hearts. With some of the heart muscle so damaged in fact that the heart now goes into a different disease process altogether known as congestive heart failure. I will explain congestive heart failure in a moment, but look at this. Look at the rise in number of deaths from congestive heart failure that are occurring in the United States. Quite the converse to what I showed you earlier. We have, in fact, an epidemic of heart failure that we now have to deal with.

Heart failure is something where the normal healthy heart, having good strong muscles that pump the blood where it is supposed to go, becomes damaged by a heart attack, or sometimes it is an inherited condition which produces damage as well, and as a result of that the walls thin down, the heart gradually goes from a football shape to a watermelon shape, and in the process it loses the ability to pump strongly.

This is a weak heart. It does not force the blood around where it should be going, fluid accumulates in the ankles, all kinds of consequences ensue, and at the end of the road there is only one lasting solution at the moment to congestive heart failure, and that is a heart transplant. You cannot reverse this process currently, so it is a very drastic condition, and it is one which exists in very large measure in the society we live in. Something like four million people in this country have heart failure of one form or another. Something like 400,000 new cases are diagnosed every year.

Here is a section through a fixed preparation of a healthy heart. You can see nice thick muscles here, the ventricles by means of which blood is pumped around. And here is an example of a heart that has gone into failure. Look at the thinning down. Look at the vacuous space here. This is nothing like the strong pumping organ that it should be.

Well, what can we do about this? There are ways that we have that are fairly high tech. We have what are called left ventricular assist devices available these days. These are electrical pumps. Sometimes they are pumps driven by pressurized air but for the most part, the electrical ones are the ones favored these days. What these do is simply take the blood from the inside of the heart, pump it out into the vessels to go around the body. In other words, it is a device that assists the left ventricle, which cannot pump hard anymore, by providing an extra pump surgically planted in the abdomen. It does not really matter where it is. It is driven by an electric motor, which has a battery that can be charged, and away we go.

These are used in this country largely as what is called a bridge to transplant. That is, they keep patients alive during a time when they are waiting for a donor heart to

become available to replace their own. As you may know, donor hearts are very hard to come by, and increasingly so, and this is a lifesaving device for patients of that kind.

In Europe they are able to leave these in for longer periods. And one astonishing recent finding is that in some patients in Germany who have been on these assist devices for perhaps as long as a couple of years, then brought in to have a new heart transplanted into them, on being taken off the assist device, their own hearts now resume strong beating. It is as if during 18 months of rest, the heart has had a chance to repair itself. Obviously there is something there that is well worthy of careful examination, and that is an area of research that is going on right now. If we could have our hearts somehow encouraged to repair themselves, that would be a tremendous way of avoiding the need for heart transplantation.

Here is a patient with an assist device. He can walk around quite comfortably. He will not run the marathon tomorrow, for example, but he does quite well.

Now to go a stage further, we are working on what will soon become a clinical entity, and that is the total artificial heart. This is where the natural heart is removed altogether and a synthetic device put in its place.

The first of these was done back in 1982 by William DeVries on a dentist who suffered from heart failure and who had an artificial heart put in place of his own. He was tethered to a huge 600 pound air compressor. It was not a very comfortable situation, and he survived about 100 days before succumbing to infections and other problems.

That was the pioneering phase. Now we are getting to a stage where we can expect to see more and more patients who will actually receive total artificial hearts. The whole heart is now replaced, not just assisted, by a device that acts as a pump driven by electricity, and this is all implanted into the patient.

This is an example of one of the prototypes of an artificial heart, which is coated in a material that makes it compatible with the body's tissues so it will not be rejected, and it is stitched on to the major vessels through these cuffs and works very well at the moment in animal models where it is being tested for reliability.

The next generation of these total artificial hearts will not be those great clunkers like the ones I just showed you but will be tiny little pumps like this. I am sure you are all aware of the size of a scalpel so you can see how small this device is. This is the type of

device that will be fitted many years down the road, probably as a replacement for a whole heart. Such very high efficiency impeller pumps will be able to take over the function of the heart itself.

But this is all at the end of the road. This is all when heart disease has manifested itself as the worst possible consequence, heart failure. We should be starting earlier in the whole process if we are going to really get anywhere, and so I want to deal with a few of the major approaches we are seeing in recent years and where we may be going with them.

Those interested in heart and vascular disease have obviously had to embrace their colleagues in the genetics department as the Human Genome Project progresses, and you will hear more about that from Francis Collins later. We find that we have an enormous amount of information on the genetic nature of some diseases, which we can presumably take advantage of in order to deal with them much earlier in the disease process. Indeed, we find that some diseases are what are called monogenic. That is, generally only one gene is involved. In cystic fibrosis, for example, that gene was discovered by Francis Collins himself and his colleagues. Muscular dystrophy is another example.

What we deal with at the National Heart, Lung and Blood Institute, however, is not so simple. Coronary heart disease, hypertension, and asthma have many more than one gene involved, and they have a progression of events where genes turn on and turn off, making the situation enormously more complex than when dealing with a single gene.

Look here at some fairly recent information in '97 from the Family Blood Pressure Program, where the Institute has been supporting research trying to find genes responsible for or at least involved in hypertension. And look at the list. And this, believe me, is very early in this research, and this is just a small fraction of the number of genes that will be involved ultimately in the process, which leads to very high blood pressure.

And there are many ways in which these genes and the knowledge of them can be used. Clearly we can do a great deal of basic biology, physiology and pathology using knowledge based in DNA, but we can also move genes between different species and into human beings. We can develop new drugs. We can do a whole plethora of things and that is the way that things are moving right now. We are very much into the domain of genetics as the basis of these diseases.

For quite a long time we have been able to grow mice, siblings from the same

litter, where one or more has been genetically modified. Here one mouse received the gene for human growth hormone, and we see the result of that in the mice that are born. These two are the same age and have the same diet and have the same conditions of life but look what the human growth hormone has done to the mouse receiving that gene. That sort of observation early on led to a clear idea that gene therapy would be where we should be going, that is using manipulations of DNA and of genes in order to cure diseases.

And, indeed, I am sure we have all heard about various forms of gene therapy that have been attempted. There have been quite a few ways in which genes have been used. These are what can be done by gene therapy, at least in theory. You can correct an inborn genetic error. That is you can take a bad gene and replace it with a good one. But you can also enhance a function. You can get a function that is only operating at a low level and give the individual a few more of the necessary genes and raise the level of function. Alternatively, you can produce a new function in an individual altogether.

Of course, here lies the ethical problem that all of us recognize that is associated with moving genes around in human beings. At what stage does it become unacceptable and immoral and unethical in a societal sense to change the nature of what we are as human beings? These are what we are wrestling with now in modern cardiovascular research as much as anywhere else. Those kinds of ethical concerns.

Gene therapy is fairly young. The early stages of research were, of course, quite a long time ago. Gregor Mendel, in Austria, working with sweet peas in 1866, laid the foundations of all this. The poor man did not know it. He died without any recognition of what later on he became famous for. Then in '53 Watson and Crick discovered the structure of DNA, the chemical that forms the genes.

And so various discoveries have punctuated this train of events until the '90s, when we have the first gene therapies attempted. The transfer of genes into humans were first done here at NIH. And now we have the first human gene therapy study for cardiovascular disease. This is familial hypercholesterolemia, where large amounts of cholesterol are deposited in arteries at very early ages and children die of heart attacks at the age of five or six.

So gene therapy has been attempted in numbers of ways. A lot of it is being attempted in the cancer area, but this was the first attempt here at NIH of a child with severe combined immunodeficiency syndrome, the so-called plastic bubble child, who

had to live completely isolated from the world around it because it had no defense mechanisms against the microbes that bombard us day by day. That was a consequence of a bad gene which, in fact, was the subject of these first experiments here at NIH in giving new genes by taking out the patient's white cells, putting the new genes into them, and putting the white cells back.

And one of these children now is able to swim in the pool and has been through kindergarten, first, second, and third grade, and is apparently quite normal but still has to have those new genes given on a regular basis. So it is not an entirely successful first attempt, but a pretty good start.

Of course, as I mentioned, the one we are interested in, familial hypercholesterolemia, is a situation where small pieces of the patient's liver can be removed. The cells from these pieces can be grown in dishes then given new genes by loading the genes into viruses, infecting the cells with these engineered viruses, and then growing them up again and putting them back in the patient.

These are genes for what is called the LDL receptor. LDL receptors are small molecules, on the surface of the liver cells, that soak up cholesterol and prevent it from forming these large blockages in vital blood vessels. But, no success so far.

We have all heard of the unfortunate circumstances that have been published extensively recently about gene therapy, particularly the death of a young patient at the University of Pennsylvania in a gene therapy experiment, and the *Washington Post* has also been quick to point out that claims for this panacea have not really been met in practice. I think what we have to recognize is that these are very early days. Things inevitably go wrong in the course of human events, but we are moving in the right direction.

As we leave the 20th century inexorably behind us, we are moving to a society and a situation and a potential for research which is far beyond and quite different from anything we have known before, and now a word or two about all that if I may.

Many of the genes that have been discovered and described in the Human Genome Project can now be taken as small fragments and spotted onto silicon chips. Here you have a group of 5,000 genes or so. These can be placed into situations where you can record whether or not the genes are turned on, or turned off, or do not work at all in various disease states. There is not time to go into much detail about this process, but

suffice it to say that a very complex picture can emerge, where over the course of time, from let's say here to here, which may in this instance be a period of weeks perhaps, certain genes, each one of which takes up a row in this matrix, are shown to be turned off, green, or turned on, red. Do not ask me why those colors are reversed by comparison with traffic lights, but that is the way it has been done. But you can see that each one of these genes—and there are thousands of them—is not just on or off all the time and, therefore, predictably involved in the course of disease, but they are on and off, and on-off, in a timed sequence. A very complex situation, indeed, begins to emerge.

Using a technique of laser capture in the microscope we can examine cells that are grown on slides or taken from tumors or from hearts or from anywhere else where you can see here the individual cells in the preparation. Through the microscope these cells are zapped by laser and stuck down and what results is a collection of individual cells, any one of which can now be removed and examined in relationship to these large gene matrices on the chips that I have been talking about.

And so in this case, Lance Liotta, who looks at prostate cancer here at the NIH, has been able to show that over the years, here a 10-year period, the cells of the ever-growing and evolving prostate cancer can be identified by these methods and examined in relationship to whether the genes are on and off, and what the proteins that are formed are doing.

But all of this, of course, is producing inordinate amounts of information. We could probably do the same kind of thing that Lance Liotta did with prostate cancer for atherosclerosis, which takes several years to develop. We could take samples at the various periods of evolving blockages in blood vessels and learn a great deal about the genes responsible for that process. So that is where we probably will be finding ourselves putting a lot of our money. But the outcome is this tremendously complicated mass of information, genes off, genes not working, genes on, and the more genes we know about, the worse it gets.

Here is a slide by Mark Boguski, from the National Library of Medicine, showing how information has increased exponentially over the recent past two or three decades. From 1965 to the present time, these curves represent the number of genes that have been identified, the number of their products, the proteins that have been characterized, the number of citations in the literature that have been made, having dealt with these. In all of these we are dealing with an onslaught of information such that biomedical research now becomes a problem of information in 2000 and the years to come. How do we deal

with that?

One way to deal with complex situations and large amounts of information is by better visualizing things. We have always had good microscopic images of things that interest us, but now we have new techniques, such as echo cardiography using ultrasound, which can be used in a variety of ways.

Here, for example, is a patient with a strange anomaly in the carotid artery, which supplies blood from the heart to the brain. This particular patient had a loop in the artery, which is quite uncommon, and this was shown without any kind of surgery by placing an ultrasound probe on the surface of the neck at the site of the artery and using echo ultrasound, based on the Doppler effect, to color-code flow in one direction as red and flow in the opposite direction as blue. So you can see the anomalous loop that this patient has inherited, which can give the surgeon and the physician very important information as to how to deal with that particular individual.

But as we proceed, we see all kinds of different ways of visualizing blood flow here in hearts. Most important these days is the use of magnetic resonance imaging. This is a rather old picture of the heart and the lungs in the chest done noninvasively. Put the patient into the magnet and produce these marvelous pictures. And we can see what is actually happening in real time as we visualize them.

Here at the extreme level of high magnification is a large molecule, acetylcholinesterase. It is important in muscular contraction. For the first time, we are able to show, using vast amounts of data by novel imaging devices, that the molecule actually splits in two at one stage in order to receive the material it is going to work on before it combines again. Nobody had the foggiest idea that that would happen.

But let me tell you that even though that particular phenomenon takes place over a period of one or two picoseconds, it took the resources of all the supercomputers in three of the nation's largest national laboratories to produce that short-lived image. So we now come up against a real barrier to progress, computing time. These are huge amounts of data that we have to deal with, and we are really not set up perfectly yet to deal with them, but we will be. We are getting there.

So what we are trying to see now and trying to deal with is a move away from the conventional biology of physics and chemistry that has driven science for so long through genetics and cell biology into an area we can call complexity, where we have to take new

approaches altogether to the study of things, where vast amounts of information are involved, and where we can no longer say we find a gene, we take a second gene and see what those two do together. That is too simplistic. We have a much more complex situation altogether.

In fact, the study of complex systems is becoming slowly but surely entrenched in all biology. The cover of the issue of *Science* shown here illustrates what can be done with something as complex as schools of fish and their movements and the prediction of where they will go by use of new mathematical approaches.

Conventional attempts to describe complex interrelationships have left much to be desired. What we see here is an attempt to take all the biochemical experiments in this area that have been performed, put them all on to one sheet of paper in the hope that you illuminate this simple process, apoptosis as it is called. It does not tell you anything. It does not tell you anything about what happens in time, nor whether some elements are operating at one moment in time and others are not. It is a static picture, and it represents the usual way of doing things. We have to take a different approach.

Einstein was one who faced complexity, faced it squarely and came up with something rather simple, $E=mc^2$. We cannot quite get as simple as that in biology. We need, in fact, a different approach in the life sciences, and what I want to finish with in the few minutes that remain is a quick look at some of the things that we might expect to be done which move us into this different domain.

You may have heard of chaos. Chaos theory is a mathematical device. It is not chaos in the sense of increasing disorder. It is a strictly mathematical computational analysis that can produce from data strange and wonderful geometric figures called fractals. If you plot the data using chaos theory you come up with these beautiful images. The reason that this has not gone very far in natural science, I believe, is that scientists have been fairly conservative and say, "Well, that is coffee table art. That is not science. Because it looks beautiful it, therefore, cannot be scientific." I believe there is a certain amount of prejudice there. But believe me, this way of representing data can be extraordinarily informative.

Here is one way of representing a mathematical set by a fractal picture, and you can see it bears a rather striking resemblance to this natural object that is found in the oceans. So we find that chaos and nonlinear dynamics exist all the way through the world. Here, believe it or not, is one way of plotting what the heart does, giving us this

beautiful fractal picture. Now you may say what possible value can that have for us with our heart diseases?

Well, the fractal images I have been showing you have what is called a scaling property. If you take a fractal picture and then remove a tiny part of it and magnify it, you find it resembles almost uncannily the first image from which it has come, and so on down through many orders of magnitude. That is the anatomy of a fractal picture.

Let's look at variability in this particular instance. If you take a small segment and expand the time scale from 300 minutes total to 30 minutes now, and then to three minutes, you get the same kind of picture repeating itself. This kind of analysis shows you what, in fact, underlies something that has chaotic properties. If you look at the heart rate you will find that that does the same, too.

Now what we are looking at here is heart rate variability. That is if you were to feel your radial pulse you could check what your heart rate is. You might say, well, it is beating 60 times a minute and that is good, and it is. That is one beat per second. But by that crude method you cannot distinguish whether it is one every second exactly or whether the time between the first beat and the second was just a little more, the first beat and the second beat was just a little less than a second, between the second and the third was a little less, and so on, so that the average is 60 beats per minute.

Here is a plot of the heart rate variability between 80 and 40 over a period of 300 minutes, and it varies this way in a healthy individual. Take a small segment. Look, it has got the same characteristics. Small segment, the same characteristics. That is the fractal pattern of a normal heart.

What good is that for us? Well, we find that this fractal pattern of a normal healthy human subject can be measured and can be plotted, but in certain patients who are about to die suddenly, when the heart loses its properties of solidly beating and forcing blood around the body, loses that property altogether and the patient drops dead on the spot, look what happens.

The variability in the heart rate is totally lost. Now we have a heart that is beating here about 90 beats to the minute absolutely rigidly. Ninety every minute without any variability between the beats. It is paradoxical. It is almost as if this should be the healthy heart and the chaotic heart should be dangerously life-threatening, but it is the reverse. So we have here the wherewithall to deal with hugely complex situations of

beating hearts by a new kind of informatics called complexity, and I think we will be seeing much more of that.

I will finish with this slide of a healthy young woman coming out of the swimming pool, creating for us a beautiful fractal pattern, but inside her is a heart and blood vessels with equally fractal properties which we hope are just as chaotic.

I have brought with me an example of a ventricular assist device, which you may like to handle. You would be surprised at how heavy it is. And also an example of the newest type of small impeller pump that might some day be an artificial heart. They are here. So please feel free to come up and examine them.

Meanwhile, if there are any questions, I will be glad to try and answer them.

This is being recorded so if you will be kind enough to speak up, hopefully it will register.

QUESTION: In seniors, what causes the systolic blood pressure to go up and not the diastolic pressure?

DR. ROBINSON: That is the first number to increase while the second number stays where it is. If you recall the origin of those numbers, the first is when the heart is pumping, and clearly when that number goes up, there is something having to do with the heart's action that is at fault. It is a complex situation that I am not really qualified to address explicitly and it will vary with different patients, but it generally indicates some aging process having to do with the heart.

QUESTION: Can congestive heart failure be reversed?

DR. ROBINSON: That is another good question. There are some situations where you can expect that you can reverse the process. Let me give you a good example from some recent work that has just been reported from France last week and it has been repeated, I think, in Pittsburgh. With hearts that are failing that are now melon shaped, they have been taking cells from the leg muscle of the same patients, and growing those in culture, then converting them so that they behave like heart cells, then putting them back into their own hearts, where they are not rejected (because they are their own tissue), and it looks as though there may be some improvement in heart function.

QUESTION: There was an NIH lecture I went to about a year and a half, two years ago, where I believe it was a Harvard Medical School researcher went through a whole series of studies with the use of anticholesterol drugs, cholesterol-lowering drugs, and his observation of further examination of the data indicated—and this is the first part of the question—indicated that there was not a strong correlation between the use of the drugs and death as a consequence of heart attack.

The second part of the question is I believe over the past couple of years there has been different items written (inaudible) the fact that measurement of LDL and HDL are not necessarily the finite evaluation, that there is another chemistry in the blood, which implies a better reading as to the effect.

DR. ROBINSON: Besides HDL and LDL, there is C-reactive protein, and there is homocysteine. These are new risk markers in the blood. There are a number of these that are coming along but they have not borne out in the public health context, nor in large populations just yet. They are only potential markers for risk of heart attack.

With regard to what is called a meta-analysis that your investigator reported, there are dangers in that inasmuch as a lot of studies are taken which have been performed under many different circumstances with different types of controls, different types of patients, kind of lumped together and reanalyzed. Now you can intuitively understand that that tends to average everything out. And you lose the fine tuning and you lose a lot of the important information. That is not to say that there was not a correct inference that could be useful. And, indeed, we now know that cholesterol is by no means the whole story. It has been an important way in which people have avoided heart attacks and atherosclerosis. It is definitely important. No question. But much more besides, including possibly viruses that cause infection and that create damage of blood vessels on top of which cholesterol problems may mount. As well as that, the immune system going out of whack and producing circumstances which tend to exacerbate the problems.

So all of these are more recent findings, none of which have yet really stood the test of time so that they have reached the clinic yet. But your observations are, in fact, valuable inasmuch as they express skepticism, which is of course the root of good research.

QUESTION: Why don't we hear much about infectious agents as the cause of atherosclerosis?

DR. ROBINSON: Because there has not been enough information to be absolutely certain that this is happening. The hypothesis that people have been working with is that herpes viruses like cytomegalovirus (CMV), which, as you may know, all of us carry all the time, may infect the linings of the blood vessels and create little sores which open the lining of the vessel to deposit of cholesterol, to immune cells, to other things, which then may go on to progress to atherosclerosis.

To prove that, you would have to go to small children, and you would have to see whether cytomegalovirus infections could produce atherosclerosis, but you cannot do those kinds of experiments obviously so you have to use circumstantial evidence. You have to take pathological specimens from children who have died and from middle-aged people who have died and older people who have died, look for the presence of the virus and its antibodies.

But none of that gives you an explanation of the mechanism. It only says the virus is there and since it is there in most of us anyway, it is a very difficult problem to solve. It is a nice hypothesis which will be extraordinarily difficult to prove, and it has not yet reached a stage where it is worth taking a risk and giving everybody antibiotics or something like that to prevent any kind of infection as a consequence of which they might be expected to have less atherosclerosis. We just do not have the information.

QUESTION: We have talked a lot about basic research and its biology, and that—for pharmaceutical companies—identified as a target is very important. And what has happened with biotechnology and robotics is pharmaceutical companies can generate an enormous number of compounds, and can test in a day or a week a quarter million in their library against targets and so that is, you know, a phenomenal increase in what can be done and then they can develop—new drugs can be developed much more quickly and then more precisely when better targets are identified.

DR. ROBINSON: That is correct.

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